12-9-2006

Effectiveness of postharvest sanitation treatments on microbial load of blueberries

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EFFECTIVENESS OF POSTHARVEST SANITATION TREATMENTS ON MICROBIAL LOAD OF BLUEBERRIES

By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Food Science and Technology
in Department of Food Science, Nutrition, and Health Promotion

Mississippi State, Mississippi

December, 2006

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Pages of study: 89

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The purpose of this study was to determine the quality and microbial load of blueberries at different maturity stages, to develop the effective microbial recovery method and to compare sanitation postharvest treatments on microbial counts of blueberries. The soluble solids and the sugar to acid ratio increased, while pH and TA decreased with maturity. Ripe berries had lower yeast and mold counts (YMC) at other maturity stages, but there were no differences on aerobic plate counts (APC). The medium pH was lower for stomaching and blending than hand massaged samples. This leads to higher recovery of microorganisms by massaging. Sodium hypochlorite at 400 ppm was effective in reducing APC but not

YMC. Acidified sodium chloride was very effective, lowering APC and YMC below detectable level. All sanitation treatment did not influence sensory attributes of blueberries.

DEDICATION

TO MY LOVELY FAMILY,

My parents, Chung-Ping Chen and Hsiu-Chen Chen Weng, and my brothers, Kuan-Jen Chen and Yu-ting Chen.

ACKNOWLEDGMENTS

First, the author would like to genuine thank Dr. Juan L. Silva for assisting as my major professor and for his outstanding ideas and his support in this thesis. Without his patience and encouragement, this thesis could not have been possible.

Secondly, the author also would like to thank the committee members Dr Mark W. Schilling, who assisted in the statistical analysis, and Dr. Frank B. Matta, who helped in the postharvest analysis experiment of this study. I also want to thank Dr. William B. Mikel as a head and graduate coordinator of the Department of Food Science, Nutrition, and Health Promotion.

Thirdly, I thank my fellow graduate students, friends and lab colleagues for their company and great help: Dr. Taejo Kim, Dr. Supanee Danviriyakul, Jelena Stojanovic, Bang-Yuan Chen, Youkai Lu, Kerri Coode, Kim Matta, Rebecca Morrison, Allessandra J. Pham (Sandy), Hye-Jin Kim, Julia Callahan, Carline, Deborah Huffman, Wei-Lien Weng, Kaewplang Chonthida (Tina), Neil Bogart,

Amy Inmon and many friends during my two years of school life.

Also, a very special thanks to Mr. Jiu-jiu Chou senior, Mr. Scott Chang senior, Dr. Kai-Ts'ung Yang, and Mr. Joseph Andol at Mississippi State University and Mrs. Candy Pierson-Charlton at Oregon State University. Without their great help and encouragement, this study would not have been possible.

Finally, I am very thankful to my loving parents Chung-Ping Chen and Hsiu-Chen Chen Weng and my brothers, Kuan-Jen Chen and Yu-ting Chen for all the encouragement and support in my life.

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CHAPTER I

INTRODUCTION

Nowadays, demanding blueberry products is in high demand due to their high antioxidant content. Hazen and others (2003) reported that many health associations report that eating at least five servings of vegetables and fruit a day is for good health. Because of these facts, blueberries are becoming more popular in modern society.

According to the North American Blueberry Council (2006), the value of fresh blueberries in the United States rose from $101 million in 2000 to $220 million in 2005. Due to their characteristics, such as respiration after harvest (climacteric), blueberries have some limitations keeping quality. Not only respiration but cross contamination, environmental conditions, unsanitary handling, and processing are also responsible for fruit deterioration (Beuchat 1998).

Schilder and others (2002) demonstrated that fruit rots are a major cause of

quality deterioration in blueberries. Anthracnose *(Colletotrichum acutatum*), alternaria (*Alternaria spp*.), and botrytis (*Botrytis cinerea*) are three bacteria that are commonly involved in blueberry decay. The National Agricultural Statistics Service of the USDA (1998) reported that more than half of the chemical treatments that were applied pre-harvest to prevent fruit rots on blueberries were in Michigan.

Many preharvest and postharvest methods have been developed and utilized to decrease decay and enhance the keeping quality of blueberries. Applying preharvest fungicides is one method that is used to prevent berry rots. Hanson and Hancock (2000) reported that Captan®(Captec® 4FL, Micro-Flow Co) has been used as a postharvest fungicide to decrease fruit rots in Michigan. Scholar (containing the active ingredient fludioxonil) is one postharvest fungicide that was approved by the United States Department of Agriculture in 2002 (Tedford and others, 2005). Postharvest treatments are important for the blueberry industry. The main reason for postharvest treatment is to control damage, and to decrease the spoilage and pathogenic microflora and dirt on the surface of fruits and vegetables (Beuchat 1995). This results in a product with extended shelf-life.

There are several different postharvest treatments used in the food industry. The most commonly used treatments are dip and spray chemical solutions used on fruits and vegetables (Crowe and others 2005).

Beuchat and others (2001) found that sanitization treatments are useful in decreasing food borne bacteria on fresh fruits. Crowe and others(2005) reported that 500-1000ppm chlorinated water spray can decrease the microbial counts in wild blueberry produces processed in Maine, such as frozen or canned blueberries. Crowe and his co-authors (2005) also showed that using a 1% hydrogen peroxide solution for 120 sec yielded lower counts of aerobic, yeast, and molds. Donahue and others (2000) stated that if treatments are useful for blueberries, both products and consumers are getting an advantage. Crowe and others (2005) also reported that besides chlorine, harvesters can apply hydrogen peroxide, ozone, citric acid, and ethanol as postharvest sanitizers. Although chlorine is a useful and common chemical sanitizer in the food industry, the unpredictable results causes some limitations such as formation of toxic by-products. (Crowe and others, 2005)

Blueberry also maturity influences the quality of the fruit. Sugar content,

acidity, antioxidant ability, and total phenolic content change during blueberry ripening (Kalt and others, 2003; Ayaz and others, 2001). In addition, it is better to pick the fruits while they are ripe (Kupferman 2006). Perkins-Veazie and Collins(1996) reported that overripe blackberries have increased decay during storage at 2℃ after seven days. There are many reports on quality of blueberries; however, very few have examined the effect of blueberry maturity on microbial flora.

Besides the issues of safety and shelf-life on blueberries, an approved standard method for determining the recovery counts of microbial load on blueberries is needed. Pathogen recovery methods on vegetables and fruits have been developed (Beuchat and others, 2001). It is not easy to find a good standard method for microbial counts due to the multiplicity of factors and products we encounter (Beuchat and others, 2001). Therefore, there is a need to develop a criterion to determine microbial counts on the surface of blueberries.

The objectives of this study were to 1) examine the quality of different stages of maturity of blueberries, 2) establish a standard preparation method for microbial

counts of blueberries, and 3) evaluate the most effectiveness of postharvest sanitation treatments on microbial load of blueberries.

CHAPTER II

LITERATURE REVIEW

Blueberries and Health

The fact that fresh vegetables and fruits are healthy for the human body is reported by many nutritionists, researchers and other scientists (Bechat and others 2001; Hazen 2003; Sellappan and others 2002; Velioglu and others 1998; Smith and others 2000). In the past decade, at least five vegetables and fruits a day is recommended by many nutritionists (Bechat and others 2001). Cao and others (1996) reported that blueberries have a higher amount of antioxidants than other fruits and vegetables. There are many reasons for production of oxidation substrates in the human body (Ames and others 1993). When oxidants are produced in the human body, the damage will induce aging and increased the risk of diseases such as cancer, heart disease, and other chronic diseases (Ames and others 1993, Moyer and others 2002, Velioglu and others 1998).

Antioxidants are compounds that can lesser and even hinder the oxidizing chain reactions and counteract free radicals in the body (Velioglu and others 1998; Prior and others 1998). Therefore, a diet of vegetables and fruits that are rich in antioxidants is one of the ways to obstruct the formation of oxidants and prevent diseases (Ames and others 1993; Moyer and others 2002; Kalt and others 1999). Besides being rich in antioxidants, blueberries are also a good source of dietary fiber and vitamin C as well as other vitamins and minerals such as calcium, iron and zinc. Moreover, blueberries are good for a person's diet because they are low in sodium and calories (Berries and wild Fruit website 2005).

Blueberry Industry in the United States

Bush Types

There are three different kinds of blueberries that grow in North America. They are highbush blueberry (*V. corymosum*), lowbush blueberry (*V. angustifolium.*), and rabbiteye (*V. ashei*) (Kalt and others 1999). Both lowbush and highbush blueberries have been commercially harvested and have been indigenous in North America for many decades (Kalt and others 2001). The major

production of lowbush blueberries is in eastern Canada and the northeastern United States (Maine and New Hampshire) (Kalt and others 2001; Strik and Yarborough 2005). On the other hand, highbush blueberries are harvested throughout the United States such as New Jersey, New York, Michigan, Arkansas, Texas, Washington, and Oregon (Kalt and others 2001; Strik and Yarborough 2005). In addition, there are southern highbush blueberries which have evolved from *V*. *corymbosum* L. introgressed with *V. darrowi* Camp. They can grow at very high temperatures and grow in Florida, Georgia, Mississippi, and North Carolina (Kalt and others 2001; Strik and Yarborough 2005). The blueberries which have the calyx on the berry that resembles the eye of the rabbit are called rabbiteye blueberries (http://www.botany.com/vaccinium.html 2006). They grow in hot, humid, and clamatorial regions like Mississippi.

Harvest Seasons

Blueberries require 120 to 160 growing days for the fruit to fully ripen (www.usbc.org 2006). In the spring, blueberry plants start to flower and are depend on bees for pollination. Due to different cultivars and weather conditions,

blueberry fruit needs about two to three months after bloom to ripen (www.usbc.org 2006).

Blueberries are ready to harvest from mid-April through early October and the biggest harvest month is in July, which is National Blueberry Month. Florida is the first to harvest blueberries in the early spring in North America, and Canada harvest in October or later (www.usbc.org 2006). Due to the location in the southern hemisphere, blueberries in Chile, South America can grow and ship to the United States in the winter. Therefore, blueberries in the low season in North America are in high season in Chile (Beaudry and others 1998) and other countries south of the equator such as Australia, New Zealand, and Argentina. Processed blueberries are also available year round for the food processing industries (www.usbc.org 2006).

Blueberry Production

The production of blueberries increases each year. Table 2.1 shows the production of blueberries in North America in the past five years from 2000 to 2005. (www.usbc.org 2005). According to their data, the value of fresh blueberries

in the United States increased from 101 million in 2000 to 220 million in 2005 (Table 2.2).

Harvest Type

When blueberries ripen enough and have a deep blue color, it is time to harvest (www.usbc.org 2006). There are two major harvest types in the blueberry industry. They are hand harvest and machine harvest. Besides the two harvest types, U-Pick is another harvest type in the blueberry industry (www.usbc.org 2006). In many small fruit industries, mechanical harvesting is popular and has been successfully used (Morris 1983). Because of increased the blueberry production, high labor costs, and less damage, growers of blueberries prefer to use mechanical harvest instead of hand harvest (Moore 1994). Moore (1994) reported that blueberries in most states that had large production such as Michigan, Florida, Georgia, and Oregon were machine harvested. However, small farmers need to offer pick all their fruit by hand.

The blueberry farmers adjust the blueberry plants that can fit into the machine to harvest when they are growing (www.usbc.org 2006). The machine is driven

through the bluebery bushes and it shakes the blueberry plants to remove blueberries from the shrubs. Blueberries will drop into buckets or conveyors from the bushes (Morris 1983; www.usbc.org 2006). In some fields, the blueberries will move to a packing house by conveyer after harvest, where the leaves and trash is removed by using high-velocity air (Morris 1983). Due to different stages of blueberry ripening, there is a need to go though the field several times. In general, blueberries are sent to packing houses or processing plants near the field to do more processing and packing, depending on market needs (www.usbc.org 2006).

Some fresh blueberries in North America are shipped to other countries such as Japan (more than 500 metric tons) and Iceland (100 metric tons) (www.usbc.org 2006). According to the US Highbush Blueberries Council (2006), 90% of the blueberry world production is from North America. Most hand picked blueberries are used for fresh market; on the other hand, processed blueberries are from machine harvest cultivated blueberries (Moore 1994). Also, wild blueberries are only used for processing no matter which harvest type is used (Moore 1994).

Changes during Maturity Stages

Size

Blueberries from the ovary of the flower are ready to eat in about three months. Eck (1988) described the characteristics of blueberry development. Blueberries are divided in three different stages. In stage I, blueberry cells divide and increase quickly. Then, blueberry growth slows down during stage II. Finally, blueberries grow rapidly to reach full maturity at stage III. Blueberries stay green for forty-five to fifty days before maturity. After that, the size of berries increases about 50% in its diameter. Kalt and others (2003) also found that blueberry size will change during different ripening stages. When blueberries reach full ripeness, they are larger than unripe blueberries (Kalt and others 2003).

Color

The color on the berries changes from green to deep purple during ripening (Eck 1988). Wills and other (1998) claim that fruit color is an important factor to help people decide if the fruit is ripe or not. Losing the green color is one of the most popular characteristics of fruits during ripening including blueberries. Color

change is due to the structure of chlorophyll abasement. Normally, when the pH value is decreased, the structure of chlorophyll will degrade (Wills and other 1998). Therefore, the color of fruit will lose its green color during maturity (Wills and other 1998). After the purple color develops, the blueberry size increases another 20% and sweetness and flavor starts to develop (Eck 1988).

Phenolics, Anthocyanins and Antioxidants

Besides size and color, the phenolics, anthocyanins, and antioxidants also change during blueberry ripening. Blueberry from pink to 50% blue may have higher anthocyanins (Kalt and others 2003). The amount of anthocyanins will be influenced by the color of the fruit (Eck1988). Kalt and others (2003) reported that anthocyanins and antioxidants did not have a positive relationship with maturity stage. Phenolics and anthocyanins of blueberries are influenced by different cultivars and the degree of ripeness, but not antioxidant activity. Anthocyanin contents were lower in the unripe stages than the fully ripe stage after harvest. However, phenolic and antioxidant concentrations decreased when blueberries attained full ripeness (Kalt and others 2003).

Although phenolics are reduced in blueberries when they are ripened on the plant, total phenolics also have a chance to increase during storage. All phenolics, antioxidants, and anthocyanins are increased after a few days of storage. Eck (1988) claimed that the maturity of fruit is related to pH value, total acidity, and soluble solids of the fruit.

Sugar and Acid

When blueberries mature, sugar content increases and titratable acidity decreases (Eck 1988). Ayaz and others (2001) added that fructose and glucose increased during berry maturity. They also found that fructose contents were higher compared with other sugars during berry maturation. They found that sucrose in blueberries was not detectable until the mid ripen and ripening stages. Citric and quinic acid are two major acids in blueberries. They decreased when the blueberries ripened.

The sugar-acid ratio was reported to increase during blueberry maturation (Eck 1988). Sugar-acid ratio is negatively correlated with shelf-life. When sugar content increases, shelf-life decreases. Also, the total acidity of blueberries

decreased and pH value increased in storage (Eck 1988). Soomro (1998) examined the quality of rabbiteye blueberries that were over 30 days at 2℃. She found that SSC does not change during storage period but SSC/TA was increased after 30 days storage (Soomro 1998). Fruit in early harvest has better quality than berries that are harvested later due to high sugar content and low acidity. The sugar-acid ratio is a good quality indicator of blueberries (Eck1988).

Respiration Rate

Respiration is one major metabolic process that happens in living plants, and fruits and vegetables after harvest (Wills and others 1998; Irtwange 2006). Respiration in fruits and vegetables is just like respiration in humans. Oxygen can be used during the processing and carbon dioxide (CO_2) will be produced during the respiration (Irtwange 2006). Respiration is also a way to degrade complex molecules such as sugar or starch into simple molecules (Wills and others 1998). Irtwange (2006) reported that fruits and vegetables lose their flavor quality and sweetness during respiration. Song and others (1992) demonstrated that respiration rate decreased while CO_2 increased in blueberries during modified

atmosphere storage. Also, variety blueberries cultivars will have a different respiration rate.

Ethylene production

Ethylene production is one way to identify climacteric fruit from non-climacteric. If fruits and vegetables are classified as climacteric, they will be exported to ethylene during post-harvest ripening as compared to non-climacteric fruits and vegetables (Wills and others 1992) Ethylene (C_2H_4) is a plant hormone that can stimulate the maturation of fruits and vegetables and even senescence in small amounts (Irtwange 2006). Poole and others (1998) state that blueberries are climacteric a fruit due to their production of ethylene and since they are limited by low temperature and controlled atmospheres. A similar finding was reported by Zheng (2005) who found that respiration rate and ethylene production can be influenced by oxygen concentration and air storage.

Fruit Decay Development

Fruit rots are the major problem to the quality of blueberries (Hanson and Hancock 2000). Many fruit-rotting fungi cause quality losses in blueberries.

Colletotrichum gloesporioides, *Alternaria*, and *Botrytis* spp cause diseases on the fruit (Hanson and Hancock, 2000; Ballinger 1983). They are influenced anytime during bloom and postharvest (Hanson and Hancock 2000). Besides fruit rots, there are many reasons that cause fruit decay such as harvest type (Morris, 1983; Miller and Smittle, 1987), maturity (Basiouny and Chen, 1998), respiration rate, storage temperature (Basiouny and Chen 1998), postharvest processing (Ballinger 1983), stem scar injury (Cline 1996), and improper handling. In order to prevent fruit decay, there is a need to know how the fruit decay is caused.

As previously mentioned, microorganisms are everywhere during postharvest and processing. Most fungi cannot grow through the skin of a wholesome fruit. However, once the skin of the fruit gets damaged, onset of decay is rapid (Wills and others 1998). Morris (1983) stated that machine harvested highbush blueberries had 44% more loss than hand pick berries.

Harvest Type and Decay

Morris (1983) reported that around 32% of machine harvested blueberries are softer than those that were hand harvested. Miller and Smittle (1987) evaluated the quality of hand and machine harvesting on rabbiteye blueberries.

They found that blueberries that were harvested by machines were less firm than hand pick berries. Also, machine harvested rabbiteye blueberries had significantly higher decay.

Maturity

Basiouny and Chen (1998) reported on the relationship between decay and maturity. They found that blueberries that were 100% blue had more decay than blueberries that had less percent blue. Also, the blueberries that were harvested earlier had less decay than that were harvested later (Basiouny and Chen 1998) Maturity is one of the factors that influences fruit decay. Wills and others (1998) reported that microorganisms cannot influence the fruit, if the fruit is fully ripe. When fruit are in inmature or overripe stages, they have less resistance to decay (Wills and others 1998).

Environment

The environment around the field and postharvest place, such as temperature and moisture content, are also important factors in the decay of

postharvest berries (Wills and others 1998). Therefore, low temperature storage (Sanford and others 1991; Basiouny and Chen 1998), and controled atmospheres (Smittle and Miller 1998; Prange and others 1994) can reduce fruit decay. Basiouny and Chen (1998) reported that blueberries remained in good condition in appearance under cold storage. The quality of firmness was reduced after 45 days storage due to metabolic processes and ripening of the fruit. When berries are overripe and stored too long, firmness will decrease and the skin of the berries will wrinkle, shriveling, and crack (Basiouny and Chen 1998). In 80% or lower storage humidity, yeasts grow easier than molds (Sanford and others 1991).

Respiration Rate & Modified Atmosphere

Blueberries have higher respiration rates under ambient conditions when compared with lower temperatures (Nunez-Barrios and others 2005). Rabbiteye blueberries that are stored in modified atmosphere had better quality than those that were stored in air (Smittle and Miller 1998). Smittle and Miller (1998) found that blueberries that were stored under atmospheres containing 15% or 20% CO_2 and 5% O_2 had increased market acceptability. Similar finding was reported by

Prange and others (1994) who also found that visible decay in lowbush blueberries was lower when they were stored at a low oxygen concentration. A 1% oxygen concentration has lower decay in lowbush blueberries when compared with other oxygen concentrations after 42 days at 0℃.

Storage Temperature

Storage temperature is directly related to microbial counts in blueberries (Sanford and others 1991; Nunez-Barrios and others 2005). When storage temperature increases, the microbial load increases. Blueberries were less firm when storage temperature was increased (Sanford and others 1991). Compared with high temperature storage such as 10 and 20℃, blueberries were the firmest when stored at 0℃. Even though blueberries were stored at 5℃, blueberries were still less firm when compared to blueberries stored at 0℃ (Sanford and others, 1991). Therefore, one can keep quality and prolong shelf-life if berries can be stored at cold temperatures (Basiouny and Chen 1998). Blueberry damage does not influence soluble solids content (SSC). However, storage temperature does influence SSC. Blueberries stored at high temperature have increased SSC.

When blueberry storage temperature is increased, the color of blueberries is significantly changed from blue to blue-red (Sanford and others 1991). Researchers suggested reducing blueberry damage from handling and processing and storing blueberries at low temperatures in order to keep good quality of blueberries (Sanford and others 1991; Nunez-Barrios and others 2005).

Processing

Morris (1983) stated that sorting, grading, and cleaning within the processing line may cause blueberries to soften and cause more decay during storage. Ballinger (1983) also mentioned that processing in packing houses after machine-harvesting can cause berry decay because rots stretch out during processing. Microorganisms are in dust, air, rain water, irrigation water, swage, soil, feces, decayed plant materials, and contact surfaces (Beuchat and others 2001), thus can contaminate fruits at many point. Also, decay is caused by people and others during postharvest including fingernail, scratches, and abrasions, rough handling, insect punctures, and cut stems (Wills and others 1998). Therefore, it is necessary to know the cause of contamination during processing.

Surface Intactness and Surface Moisture

Cline (1996) mentioned that when berries were broken, conditions were good for *Alternaria* growth. Compared with *Alternaria*, *Colletotrichum gloeosporiodes* could grow any time during postharvest and after postharvest. If berries are wet, it provides good condition for mold to grow. Ballinger (1983) indicated that both wet and dry blueberry surfaces will stimulate *C. gloeosporides* growth during or after blueberry harvest. Cline (1996) suggested that the fruit be kept as dry as possible and handling surfaces kept as clean as possible. *Alternaria tenuissima* grow easily when berries have a stem scar (Cline 1996). It is easier to grow molds with wet stem scars than dry stem scars.

Methods of Fruit Decay Prevention

Miller and others (1984) suggest that cultivar choice, temperature of precooling, storage, and shipping are the main factors that keep quality of blueberries during shipping.

Non-chemical Treatments

Different cultivars have different degrees of decay. ‘Ivanhoe’ had significantly more decay than ‘Bluecrop’ during 15 days of storage (Beaudry and others 1998). Although the degree of maturity may influence the quality of blueberries, these difference cause to exits after two weeks storage. Immature blueberries become fully ripened after 18 days, with no apparent difference. Storage temperature will influence quality of blueberry cultivars. Storage temperature had less effect on ‘Bluecrop’. Blueberries stored at 20℃ and 10 fold increased decay with compared to thase stored at 3℃, after 30 days (Beaudry and others 1998). Miller and others (1984) reported that “Woodard” blueberries had less decay than “Tifblue” at 1℃ during two weeks storage.

Miller and others (1984) reported that there is glucose, fructose, and a small amount of sucrose in blueberries. During storage, the sugar concentrations decreased (Miller and others 1984). Blueberries that were over wrapped with plastic film had less weight loss than blueberries that were not wrapped (Miller and others 1984). Miller and others (1984) suggested that it is important to let growers know that proper storage at low temperatures can maintain the quality of

blueberries.

Chemical Treatments

Both pre-harvest and post-harvest methods have been developed and utilized to decrease decay and increase the keeping quality of blueberries. Both growers and consumers benefit from these methods (Kader 2003).

Pre-harvest Treatment

Various pre-harvest treatments are used in the fruit industry. Wills and others (1998) claim that chemical fungicides are commonly used preharvest to reduce fruit decay. However, some fungicides are used postharvest. Table 2.3 shows some pre-harvest and postharvest fungicides that are used on vegetables and fruits. The National Agricultural Statistics Service at USDA (1998) reported that more than half of the chemical treatments were applied to prevent fruit rot on blueberries grown in Michigan. Fungicides in Michigan are applied by many kinds of different ground equipment and fix-wing airplanes (Hanson and Hancock 2000).

Although postharvest and preharvest fungicide treatments have many

advantages in regards to reducing yeast and mold counts on vegetables and fruit, there are some concerns about using fungicides as preharvest treatments. Since fungi become more resistant to fungicides and the presence of chemical residues from the fungicides on the fruits and vegetables (Wills and others1998).

Post-harvest Treatment

The food industry should develop methods for postharvest treatments in order to reduce bacteria and yeast and molds counts on fruit and vegetable surfaces (Beuchat and others 2003). Sy and others (2005) reported that a reduction of *Salmonella* and yeast and mold counts after gaseous ClO_2 treatments on fresh fruits. A good postharvest fungicide should be water soluble, safe for the human body, not affect fruits and vegetables themselves, not cause visual residues, and clean the fruit and vegetable surface. However, there is no fungicide that meets all of these of requirements (Wills and others 1998). There is a need to develop postharvest treatments with chemical agents' instead of fungicides due to safety concerns, residual activity of fungicides, and the shelf-life of treated vegetables and fruits (Wills and others 1998).

Postharvest treatments are important for blueberry production. Due to labor costs, machine harvest is increasing in popularity/use. Berry rots influence the market acceptability and shelf-life of blueberries (Ceponis and Cappellini 1978). There are so many reasons that postharvest treatments are necessary for the food industry, but the main reason for postharvest treatments is to control damage and to decrease the pathogenic bacteria and dust on the surface of fruits and vegetables. The common postharvest treatments on vegetables and fruits are show in Table 2.4. These treatments can decontaminate/eliminate and prolong their shelf-life, giving consumers good quality products. There are several different postharvest treatments used in the food industry. The most common methods are to dip or spray chemical solutions on fruits and vegetables (Beuchat and others 2001; Crowe and others 2005). Ceponis and Cappellini (1978) reported that berry rots were decreased after several postharvest treatments such as Captafol®, 2-aminobutane, sodium hypochlorite, and chlorothalonil. In the fruit and vegetable industry, sodium hypochlorite is a common sanitizer used in fruits and vegetables. Ceponis and Cappellini (1978) reported that sodium hypochlorite can decrease the amount of berry rots, but it also influences the surface of fruit

such as bloom loss.

Beuchat and others (2001) found that sanitization treatments were useful at destroying food borne bacteria on fresh fruits. Crowe and others (2005) reported that the wild blueberry industry in Maine determined that 500-1000 ppm chlorinated water spray decreased microbial counts in blueberry products such as frozen or canned blueberries. Donahue and others (2000) claimed that if treatments were useful for blueberries, both producters and consumers were getting an advantage.

Crowe and others (2005) also reported that besides chlorine, packers applied hydrogen peroxide, ozone, citric acid, and ethanol for postharvest treatments as sanitizers. Although chlorine is a useful chemical sanitizer in the food industry, unpredictable results can cause some limitations such as the formation of toxic by-products (Crowe and others 2005). They added that 100 ppm chlorine could reduce aerobic and yeast and mold counts on lowbush blueberries, when compared to citric acid and distilled water. Hydrogen peroxide may have good antimicrobial activity in some vegetables and fruits, but it will cause color changes on blueberries due to surface oxidation. Total aerobic, and yeast and mold counts

were reduced more when lowbush blueberries were treated with 1% hydrogen peroxide for 120 sec by spraying. Moreover, some fresh and low bacteria count berries maybe contaminated from other blueberries by ineffective Captan® treatment solutions during postharvest processing (Ballinger 1983).

Captan® is a fungicide that has been used to decrease fruit rots preharvest or postharvest (Hanson and Hancock 2000; Ballinger 1983; Silva and others 1987). Ballinger (1983) found that 100 ppm or more Captan® reduced yeast and mold counts in blueberries. Also, Captafol is a useful postharvest treatment for the control of *C. gloeosporides*. In addition, Scholar® and Captafol® are new fungicides that were approved by the EPA and the United States Department of Agriculture in 2002 (Tedford and others 2005).

Some fruit with crown in their bodies may not be able to use postharvest treatments with sanitizer solutions because the crowns of the fruit get wet during postharvest treatment. Then, molds and pathogenic bacteria on the crown will re-grow and cause fruit decay (Tedford and others 2005). Therefore, Ballinger (1983) suggested that it will be better to treat with a fungicide if blueberries are wet.

Captafol® (Ortho Difolatan 4F) can be used in the processing line at the control washing, sorting, grading and packaging steps. Captafol® is a good fungicide for controlling the *Colletrichum gloeosporioides* (Ballinger 1983). Ceponis and Cappellini(1978) found that 1000 and 5000 ppm Captafol® (cis-N-4-cyclohexene-1,2-dicarboximide) could be used as a fungicide to spray on blueberries to prevent rot. Ceponis and Cappellini (1978) suggested that fungicide treatment should be used after the fruit is picked to prevent stem scar and reduce fruit rots. Ceponis and Cappellini (1978) found that *Alternaria* easily caused serious fruit rots when compared to *Anthracnose* and gray molds. Ceponis and Cappellini(1978) reported that blueberry rots can be reduced by chemical treatments. Comparing different concentrations of fungicide treatment, 150 ppm Imazalil was the most useful fungicide treatment in decreasing yeast and mold counts in sweet cherries (Yaman and Bayndrl 2001). Tedford and others (2005) reported a decrease in gray mold when Scholar® was used. The gray mold, *Botrytis cinerea*, fruit was decreased from 30% to 5% fruit losses on pomegranate fruit (Tedford and others 2005). Tedford and others (2005) found only a 5% fruit loss after using a fungicide compared to 30% fruit loss without using a fungicide.

Effects of Sample Preparation Method on Microbial Counts

Raw fruit and vegetables can be contaminated anywhere in the chain of production and distribution. Once the right temperature is reached, the microorganisms will grow (Beuchat and others 2003). Some researchers point out methods to prevent microorganisms' growth, while others are developing useful chemical treatments to inhibit microorganisms on fruits and vegetables (Beuchat and others 2001). There are many factors that influence the results of experiments such as inoculum growth conditions, preparation of inoculum, method for inoculation, treatments, processing, and storage. There is a need to develop standard methods to enumerate the number of microorganisms are fruits and vegetables (Beuchat and others 2001).

Sampling methods are important not only in the food industry but also for food microbiologists (Seo and others 2003). Because of differences in fruits, such as surface and surface smoothness, the way to prepare samples for microbial recovery has to be considered (Koseki and others 2004). Koseki and others (2004) compared homogenization and swabbing on strawberries and cucumbers. They found that homogenization had higher bacteria counts than swab. A similar finding

was reported by Seo and others (2003), who found that different homogenization methods such as hand massaging, hand stirring, stomaching, and electric blending had different effects on the recovery of *Salmonella* from eggs. They concluded that manual methods such as hand massaging and hand stirring had higher *Salmonella* recovery when compared to mechanical methods.

Comparing blending and stomaching methods for sampling preparation, blending had a higher recovery rate of five native microflora (Ukuku and Fett 2004). When recoverying *L. monocytogenes* from lettuce, there was no significant difference between soaking, stomachating and homogenating in water for five minutes (Burnett and others 2004). Therefore, recovery of bacteria may differ by wash solution, stomaching, and homogenization. However, homogenization washing solution and stomaching had similar recovery of *Salmonella* on fruits including blueberries (Burnett and Beuchat 2001).

Different inoculation methods also affect bacterial counts. Lang and others (2004) found that dipping had significantly higher *E. coli* O157:H7 counts from lettuce in treatment solution than spot and spray. Koseki and others (2003) also found that when comparing dipping and spotting, inoculation on methods spotting

had higher *E. coli O157:H7* and *Samonella* counts.

In addition, the way to report the results such as CFU/g or CFU/cm^2 will influence the interpretation of the data (Beuchat and others 2001). Fruits and vegetables have so many uneven shapes. For example, weight (g) and surface (cm^2) of vegetables and fruits affect the results (Beuchat and others 2001). Bacteria counts in whole melon (log CFU/cm^2) and fresh-cut pieces (log CFU/g) are different (Ukuku and others 2004). How to compare and sample in equal and correct ways is important for many researchers. Ukuku and others (2004) claimed that mesophilic aerobes and yeasts and molds had higher counts in whole melon when compared to fresh-cut pieces.

Table 2.1 Harvest acreage, yield, production, and utilization of blueberries from 2000 to 2005 in the United States

Year	Area Harvested	Yield Per Acre	Production		Utilization	
			Total	Utilized	Fresh	Processed
	Acres	Pounds	1000 Pounds	1000 Pounds	1000 Pounds	1000 Pounds
2000	40,320	4,500	185,340	181,620	77,820	103,800
2001	40,580	4,830	200,210	195,840	88,990	106,850
2002	41,180	4,590	192,140	188,830	99,680	89,150
2003	40,970	4,580	189,650	187,700	104,620	84,280
2004	44,430	5,120	228,880	227,570	124,550	103,020
2005	48,310	4,810	233,030	232,550	117,850	114,700

Source: North American Blueberry Council (2006)

Table 2.2 The value of blueberry production from 2000 to 2005 in the United States. Unit: 1000 Dollars

Year	Fresh	Processed	Total
2000	101,101	75,470	176,571
2001	109.088	56,150	165,238
2002	141.114	53,018	194,132
2003	155,715	65,035	220,750
2004	193,058	82,905	275,963
2005	219,961	103,827	323,788

Source: North American Blueberry Council (2006)

Table 2.3 Pretharvest and postharvest fungicides that are used on the vegetable and fruit industries

	Fungicides	Product	Target YMC	References
Preharvest	Fenbuconazole(Indar 75 WSP)	Blueberries	Monilinia	Scherm and Stanaland, 2001
	SADH-succinic acid-2,2-dimethylhydrazide	Blueberries		Ismail, 1973
	Isothiocyanates		Phytopathogenic fungi	Tiznado-Hernandez and Troncoso-Rojas, 2006
	Natural volatile compounds	Strawberries, blackberries, and Grapes	Gray molds	Archbold and others, 1997
	Benzimidazole	Bananas		Niranjala Perera and Karunaratne, 2001
	Thiophanate methyl	Citrus Fruit	Green molds	Smilanick and others, 2006
	Iprodione	Kiwifruit	*Botrytis Cinerea*	Pyke and others, 1994
	Foliar, Captofol, Iprodione, and Copper	Citrus	*Alternaria*	Timmer and others, 2003
	Captan	blueberries		Silva and others, 1987
	Sportak®	Avocado	*C. acutatum, Antracnose*	Everett, 2002

Table 2.3 (Continued) Pretharvest and postharvest fungicides used in the vegetables and fruits industries

	Fungicides	Vegetables and fruits	Target YMC	References
Preharvest	Imazalil	Cherries		Yaman and Bayndrl, 2001
Postharvest	Scholar(Fludioxonil)	California Pomegranate	(*Botrytis Cinerea)*	Tedford and others, 2005
	Imazalil	Lemons		Schirra and others, 1996
	Calcium Chloride	Peaches and nectarine	*Rhizopus stolonifer*	Tian and others, 2002

Table 2.4 Postharvest antimicrobial treatments on vegetables and fruits

Vegetables and fruits	Treatments	concentration	References
Lettuce and parsley	Chlorinated water	200 ppm	Lang and others, 2004
Blueberries	Chlorine	100 ppm	Crowe and others, 2005
Wild blueberries	Chlorine	3.8mL/2 Liter	Hazen and others, 2003
Lettuce leaves	Chlorine Dioxide gas		Lee and others, 2004
Fresh cumbers	Chlorine Dioxide		Costilow and others, 1984
Fresh cut vegetables and fruit	Gaseous chlorine Dioxide		Sy and others, 2005
Strawberries	Batch+ Chlorine Dioxide gas		Han and others, 2004
Blueberries	Captafol	100 ppm	Ballinger, 1983
Blueberries	Control Atmosphere		Saltivit and Ballinger, 1983
Blueberries	Modified atmosphere		Song and other, 1992
Strawberries	Hot water		Woods and others, 1998
Strawberries	Hot water dip + biological control + CA storage		Wszelaki and Mitcham, 2002
Sweet corn	Irradiated shrink-wrapped	1.0 kGy	Deak and others, 1987
Blueberries	Irradiation for quarantine treatment	0.5~1.0 kGy	Miller and McDonald, 1996
Strawberries	Ozone	3 ppm	Rodgers and others, 2000

Table 2.4 (Continued) Postharvest antimicrobial treatments on vegetables and fruits

Vegetables and fruits	Treatments	concentration	References
Whole apples	Ozone	3 ppm	Rodgers and others, 2004
Whole lettuce	Ozone	3 ppm	Rodgers and others, 2004
Cucumbers	Ozone	3 ppm	Sko and Chu, 2001
Blueberries(lowbush)	H_2O_2	0.5%	Crowe and others, 2005
Strawberries	H_2O_2	3%	Yu and others, 2001

CHAPTER III

MATERIALS AND METHODS

Experiment l: Quality and Microbiological Counts of Blueberries at Different Maturity Stages

Sample Collection

Blueberries were picked from Reese Orchard in Sessums, Mississippi on July, 2004. Different maturity stages of blueberries came from three different blueberry trees (replication-blocks) of the same cultivars 'Tifblue'. The blueberries were picked by hand using sterile gloves. The maturity of the berries was identified visually. Green berries were light green, red berries were dark red, and ripe berries were dark blue in color and had smooth skin on the fruit (Eck 1988; Kalt and others 2003). Overripe berries had wrinkles on the skin of the berries and the calyx of the berry was dried. The sorted blueberries were placed into Ziploc® bags (S.C. Johnson& Son, Inc, Racine, WI) after picking, and stored in an ice box filled with ice. After harvest, the blueberries were taken to the MSU Department of Food Science laboratory directly for analysis within 48 hours.

Chemical Analysis

Soluble Solids Concentration (°Brix)

The soluble solids concentration of blueberries was evaluated using a Refractometer (Bauch & Lomb, Rochester, NY). The temperature was maintained at 21° with cold water circulating through the refractometer. The refractometer was calibrated using distilled water before each reading. After calibration, one drop of filtered blueberry juice was placed on a glass prism. The results were recorded and expressed as °Brix (Tokitkla 2004).

Titratable Acidity(TA)

Blueberries were homogenized using a commercial blender (Black&Decker® Handy Chopper) for three minutes. Five grams of blended blueberries were stirred with 95 ml of distilled water in a 150 ml beaker. Three drops of phenolphthalein were added as an indicator, and 0.1 N NaOH (Fisher Scientific Co., Fair Lawn, NJ) was used to titrate the sample to an end point of pH 8.2 (AOAC, 1990). The titratable acidity was represented as percentage of citric acid/100 mL of blueberry fruit. Titratable acidity was calculated as citric acid, where the meq factor used for citric acid was 0.070 (Woods and Aurand 1977):

$$TA = \frac{(\text{mL base})(0.1\ \text{NaOH})(\text{meq}=0.070) \times 100}{\text{Sample weight (5g)}} = 0.7\ (\text{ml NaOH})/5 \qquad (1)$$

pH

The pH was determined by using 10 ml of homogenized blueberries with a pH meter (Thermo® electron Corporation, Bevely, MA.). The pH meter was calibrated using two buffer solutions, pH 4 and pH 7 (Soomro,1998). pH was measured in triplicates for each sample at 22℃.

Soluble Solids Concentration to Titratable Acid Ratio (SSC/TA)

Soluble solids concentration to titratable acid ratio was obtained by dividing SSC by TA for each sample (Soomro, 1998).

Physical Analysis

Color

The color of whole blueberry samples was evaluated using a Labscan Model 6000 0/°45 Spectrocolorimeter (Hunter Associates Laboratory, Fairfax, VA). The instrument was calibrated with two standard tiles (black and white) using a quartz-halogen lamp. Each blueberry was placed on a 10 mm diameter port. Three readings were taken on each sample after rotating the berry. Ten berry fruits were used in each replication. The reflectance values of 'L' (brightness), 'a' (redness+/greenness-), 'b' (yellowness+/blueness-) were measured. Hue angle value ($\tan^{-1}b/a$), and chroma or saturation index ($SI=(a^2+b^2))^{1/2}$, and the total color difference $\Delta E=(\Delta L^2+\Delta a^2+\Delta b^2)^{1/2}$ were calculated according to Stojanovic 2003.

Microbial Analysis

Sampling Preparation

A sample of 25 g of berries was placed into a stomacher bag (Nasco whirl-pak$_{TM}$. U.S.A.), to which 225mL of 0.1% sterile peptone water was added and stomached in a Stomacher (Sweard Medical Limited London, UK) for 30 seconds (Tokitkla 2004).

Yeast and Mold Counts

Yeast and mold counts (YMC) were determined by the spread plate method by using 0.1 mL of the sample dilution. Potato Dextrose Agar (Oxoid LTD, Basingstoke, Hampshire, England) acidified with sterile tartaric acid (10%) was used as the growth medium (American Public Health Association, 1992). The plates were duplicated from each dilution and incubated in a low temperature incubator (NAPCO® Modl 4100 CO_2 incubator, Precision Scientific, Illinois, USA) at 20℃ for four days. The colonies were counted and the microbial counts were represented by log cfu/g of sample.

Aerobic Plate Counts

The total aerobic plate counts (APC) were determined by the spread plate method by using 0.1 mL of sampling dilution (APHA, 1992). Plate Count Agar was used as the growth medium (Difco, Becton, Dickinson, USA). The APC plates were duplicated from each dilution and incubated in a Incubator (Precicion Scientific, Illinois, USA) at 37℃ for 48 h. The colonies were counted and the microbial counts

were reported as log CFU/g of blueberries.

Experimental Design

A Completely Randomized Block Design with four different stages as four treatments (Green, red, ripe, and overripe) and three trees as replications were conducted (blocks). All data collected were analyzed by using the General Linear Model (GLM) procedure (SAS, 2001). The differences between means were determined using Duncan's multiple range test at $p \leqq 0.05$ (SAS, 2001). The significant test level was performed for different maturities. Statistical analysis was conducted with SAS version 9.1(SAS, 2001)

Experiment Ⅱ: Effect of Sampling Dilution and Preparation on Yeast and Mold Counts and Aerobic Plate Counts of Blueberries

Sampling Preparation

In this experiment, fresh and frozen blueberries were purchased from local grocery stores in Starkville, Mississippi on January, 2005. The fresh berries were stored at 4℃ prior to experimentation. The frozen berries were thrawed overnight before this experimentation. Each company or farm represents one replication (block). Three replications (block) were used in this experiment.

Microbiological Analysis

The microbiological analysis of different dilutions and sampling preparation were

investigated (Fig.3.1). There were two different dilutions: 1:3 and 1:10. Six 10 g samples of blueberries from the store were weighed. Three 10g samples were added into 20 mL 0.1% peptone water for 1:3 dilutions. The other three-10g samples were added into 90 mL of 0.1% peptone water for 1:10 dilutions. From each of the dilutions, samples were mixed by three methods: massaged by hand shaking for 30 sec, stomached by Stomacher (Sweard Medical Limited London, UK) for 30 sec, and blended by blender (Polytron® Brinkmann Homogenizer, Switzerland) for 30 sec. Before blending, the homogenizer was sprayed with 70% alcohol. Then the homogenizer was rinsed with 0.1% sterile peptone water to make sure that the homogenizer was thoroughly clean. Serial dilutions were made in 0.1% peptone water.

Subsequently, 0.1 mL of the suspensions was spread on the surface of duplicate plates of appropriate mediums. Plate count agar (Difco, Becton, Dickinson, USA) was used to enumerate total aerobic plate counts (APC). Colonies were counted after incubation at 32℃ for 48 hours. Potato dextrose agar (Oxoid LTD, Basingstoke, Hampshire, England) added with 10% tartaric acid (APHA 1992) was used to enumerate yeast and molds (YMC). Yeasts and molds were enumerated after incubation at 20℃ for four days. Colonies were recorded as colony forming units/g of blueberries (CFU/g) (Tokitala, 2004).

Experimental Analysis

The experiment was a two way factorial in a completely randomized block design

with two dilutions and three sampling preparations (Stomaching, Massaging, and blending). All data collected were analyzed by using the General Linear Model (GLM) procedure and ANOVA procedure (SAS, 2005). The differences between means were determined by using Duncan multiple range test at $p \leqq 0.05$ (SAS, 2005). Statistical analysis was conducted with SAS version 9.1(SAS, 2005).

Experiment lll: Effect of disinfection treatments on fresh blueberry microbiological count and quality

Sampling Preparation

Fresh blueberries from three different companies (blocks) were purchased at the local supermarkets in Starkville and Jackson, Mississippi on September, 2006. Blueberries were transported to the MSU Department of Food Science laboratory and stored in a 1℃ refrigerator (Admiral Company, Galesburg, USA) until needed (two to five days).

Before the sanitation experiment, the moldy and immature blueberries were sorted out with sterilized stainless nails. The berries that came from the same company were mixed together.

The sanitation treatments that were used for this experiment were Scholar® fungicide (4-(2, 2-difluoro-1, 3-benzodioxol-4-yl) -1H- pyrrole-3– carbonitrle) from Syngenta Crop Protection, Inc. (Greensboro, NC. USA.), and chlorine (Clorox®

regular bleach 5.7% available chlorine, as sodium hypochlorite,The Clorox Company, CA. USA)(200 and 400 ppm). Tap water was used as the wet control. Untreated, unwashed berries were also analyzed as a dry control.

After sorting and grading, the blueberries were treated with either tap water or one of treatments (Figure 3.2). Treatments were applied to blueberries by dipping them in each of treatment solution for 30 sec. Blueberries that were treated with either 400ppm chlorine and Scholar® were followed by rinsing with tap water. The berries were drained and divided into two parts. Each part was approximately 100 g of blueberries. One part of the blueberries was placed in to Ziploc® bags (S.C. Johnson& Son, Inc, Racine, WI), stored at 4□ overnight, and microbial analysis and sensory evaluation was performed the next day. Another part of the fresh berries were placed in Ziploc Frozen Bags (S.C. Johnson& Son, Inc, Racine, WI) at -4□ for seven days. After seven days, blueberry samples were thrawed overnight for microbial analysis and sensory evaluation. Three replications (blocks) for each process were provided.

In a separate experiment, blueberry samples were treated with 600 ppm acidified sodium chloride (Ecolab®, USA) for 30 sec. Treated and untreated berries(two replications) were placed in plastic clamshell boxed and stored in an ice box. Berries were delivered to MSU lab for microbial analysis within 48 h.

Microbiological Analysis

Aerobic Plate Counts (APC) and Yeast and Mold Counts (YMC) were determined by using the methods delineated previously (experiment II). The APC and YMC were

indicated as log cfu/g of sample.

Sensory Evaluation

Sensory evaluation of frozen and fresh blueberries was performed by a ten member panel who are familiar with blueberries and sensory evaluation techniques. The frozen blueberries were thawed and held at 4□ overnight before sensory evaluation. Three berries from each sample were placed into Sweetheart® plastic portion cups with lids (Sweetheart Company INC. Owings Mills, MD. USA) (Ottawa, 1637).

The panelists received four samples and one standard (dry samples) each time. The berry samples were evaluated for appearance, skin color (change, fade), off-odor, off-flavor, and overall quality. Each sensory property was rated by a five point descriptive evaluation. The descriptive evaluation was conducted if there were differences from the standard samples (Figure3.3). Blueberry samples were coded by three digit random numbers. Samples (5 to 10 per day) were presented to panelists on white paper in a laboratory.

Experimental Analysis

The experiment was a randomized completely block design with five different treatments (four postharvest treatments and one control). All data collected were analyzed using the General Linear Model (GLM) procedure (SAS, 2001). The differences between means were determined using Duncan's multiple range test (p□0.05). The significant test level was performed for different maturities. Statistical analysis was conducted with SAS version 9.1 (SAS, 2005).

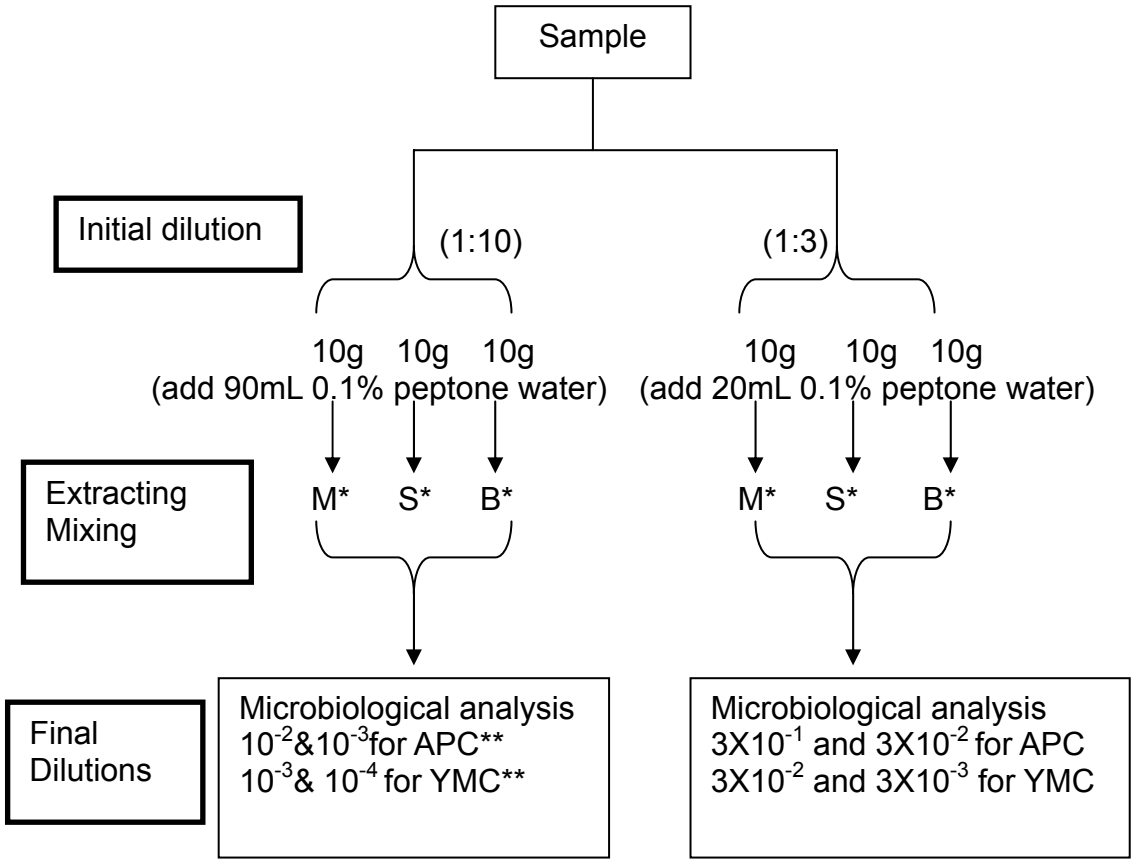

Figure 3.1 Flow chart of different dilutions and microbiological preparation of blueberries (Experiment II) (*M-Massaging, S-Stomaching, B-Blending, **APC-Aerobic Plate Count, YMC-Yeast and Mold Counts)

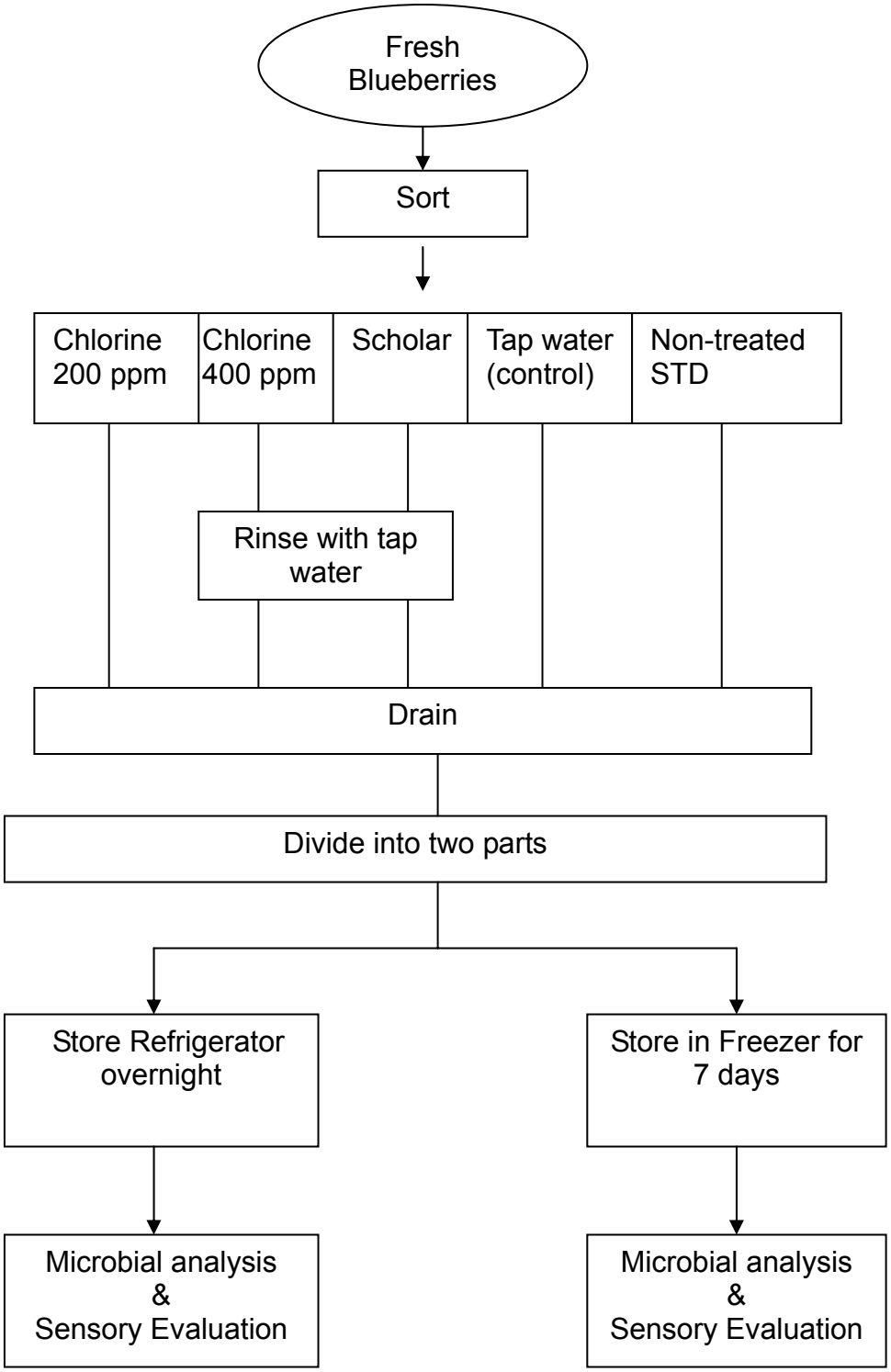

Figure 3.2. Flow chart of different treatments of blueberries (Experiment II)

Difference on Blueberries*

Name _____ Date _____

You are receiving 5 samples of blueberries to determine appearance, Skin color, Off-odor, off-flavor and overall quality. Determine and rate each characteristic.

How they are different (Is there any difference)??

Sample NO.	426	659	149	212
YES				
NO				

1. What is the difference in Appearance?

Sample NO.	426	659	149	212
Shrink				
Slightly shrink				
Moderate firm				
Much Firm				
Extreme plump, Firm				

2. What is the difference in Skin Color (change, fade)?

Sample NO.	426	659	149	212
Red Color				
Slightly Red color				
Moderate deep purple				
Much deep purple				
Extreme Deep purple				

Figure 3.3 Sensory evaluation sheet used to rate appearance and skin color of different fresh or frozen blueberries

3. What is the difference in Off-odor (Smell)?

Sample NO.	426	659	149	212
Chemical				
Slightly chemical				
Moderately fresh and none				
Much Fresh				
Extreme Fresh, none				

4. What is the difference in Off-flavor (Eat)?

Sample NO.	426	659	149	212
Spoiled, Chemical				
Slightly Spoiled, chemical				
Moderately Spoiled, chemical				
Much fresh, sweet				
Extreme Fresh, sweet				

5. What is the difference in Overall Quality?

Sample NO.	426	659	149	212
Dislike extremely				
Slightly dislike				
Moderately like				
Much like				
Like Extremely				

Figure 3.4 Sensory evaluation sheet for Off-odor, off-flavor and overall quality on fresh and frozen blueberries

CHAPTER IV

RESULTS AND DISCUSSION

Experiment I: Quality and Microbiological Counts of Blueberries at Different Maturity Stages

Data for some additional experiments are not approved directly to the objectives of the study are shown in the Appendix (Table A.1 to A.5)

Chemical Analysis

All Hunter color values were effected by maturity stages, except ‘L’ and ‘Hue’ (Table 4.1). The ‘a’ value was grater at maturity stages red and did not differ among the remaining stages. Ripe and overripe blueberries did not differ ($p>0.05$) in ‘b’ value, but there were differences ($p\leqq0.05$) between green and red berries. Green and red berries did not different ($p>0.05$) in ‘ΔE’ and chroma values. Also, red, ripe and overripe berries were not different ($p>0.05$) in ‘ΔE’ value.

Not only size changes but also color changes can be used to identify if blueberries are ripe or not (Wills and others 1998). When the green color turns to red, the chlorophyll structure starts to degrade. Moreover, the pH value causes the change of the chlorophyll structure (Wills and others 1998). The same results can be seen in this study. There was a large variability (CV) in color value of blueberries, indicating a large difference between samples (uneven color of berries). The color of berries at each stages of maturity is shown in Figure 4.1. Hue angle for green berries was about 100° (Yellowish) and decreased to about 30° (Red) for red berries; and then to about 310° (purple) for ripe and overripe berries. Chroma was high for green and red berries, but very low for ripe and overripe berries (Table 4.1).

Physical Analysis

Values for soluble solids content (SSC) differed ($P<0.05$) by maturity stage (Table 4.2). Soluble solids content was higher in red, ripe, and highest ($p\leqq0.05$) in overripe blueberries, respectively. This trend is similar to that reported by Perkins-Veazie and others (1996). They found that soluble solids content was

higher in mature blackberries than immature blackberries. Ayaz and others (2001) also indicated that fructose and glucose increased with maturity stage of blueberries. Wills and others (1998) indicated that fruit became sweeter during maturation, due to breakdown of carbohydrate polymers. Normally, protopectin can breakdown to small molecules that are more soluble in water, during maturity and ripening (Wills and others 1998).

Values of titratable acidity (TA) were different ($p<0.05$) at different maturity stages (Table 4.2), but not between ripe and overripe berries. The TA was highest ($p \leqq 0.05$) in immature berries. Ayaz and others (2001) found that immature blueberries had higher total acid compared with ripe berries. Because of the metabolism in blueberries themselves, acid decreases due to the energy needs during maturation (Wills and others 1998). This may be a reason that there was no difference ($p>0.05$) between ripe and overripe berries.

Values of SSC/TA ratio were different ($p<0.05$) among the four different stages (Table 4.2). The SSC/TA ratio in green berries was lower than in overripe and ripe berries. This is reasonable due to the soluble solids content increase and titratable acid decrease during maturity. Perkins-Veazie and others (1996)

reported that overripe blackberries have higher SSC than unripe berries. Soomro (1998) reported that SSC/TA ratio of blueberries 'Tifblue' increased during 30 days storage period at 2℃. However, Tolitkla (2004) found that SSC/TA ratio was decreased during 20 days of storage period at 2℃, and increased after 29 days under SO_2 storage due to low decay, high acidity and anthocyanin content. Basiouny and Chen (1988) reported that SSC/TA increased from unripe to overripe during 45 days storage in rabbiteye blueberries. Overall, high SSC/TA ratio is better in respect to blueberry quality due to high soluble solid content and low acidity.

Green berries had a higher ($P<0.05$) pH value than berries at other stages which did not differ (Table 4.2).

Microbial Analysis

Aerobic plate counts (APC) and yeast and mold counts (YMC) in different maturity stages of blueberries are shown in Table 4.3. There was no differerence ($p>0.05$) in APC among maturity stages. However, YMC were different ($p<0.05$) at various maturity stages. Overripe blueberries were higher ($p<0.05$) in YMC than

others. Ripe blueberries had lower ($p<0.05$) YMC than green and overripe berries. Ripen berries has lower microbial may be due to the antimicrobial compound such as anthyocyanins produce after berry ripe. Kalt and others (2003) reported that highbush blueberries in ripe stage have higher antioxidants, anthocyanins, and phenolic content than other stages. Basiouny and Chen (1988) also found that overripe rabbiteye blueberries had more decay than the ripe and unripe blueberries. APC and YMC in blueberries ' Tifblue' was increased after storage at 2 ℃ (Tolitkla 2004). Kupferman (2006) claimed that it is better to harvest fruit by ripening stage. It is easy to cause decay in overripe fruit. Decay may be caused by fungus during the growing season and the infection of immature green berries (Cline 2006).

Experiment II: Effect of Sampling Dilution and Preparation on Yeast and Molds Count and Aerobic Plate Counts of Blueberries

Microbial Analysis

The results of yeast and mold count (YMC), aerobic plate counts (APC), and pH values at different sampling methods (stomaching, massaging, and blending)

of fresh and frozen blueberries and different serial dilutions (1:3, 1:10) are shown in Tables 4.4 and 4.5. Aerobic plate counts among stomaching, massaging, and blending at both dilutions were not different ($p>0.05$) (Table 4.4). However, massaging in fresh berries was higher ($p\leqq0.05$) using APC for 1:10 dilution but not ($P>0.05$) for YMC. On the other hand, frozen berries using stomaching had higher ($p\leqq0.05$) YMC but not APC. Seo and others (2003) found that hand massaging had higher populations of *Salmonella* than stomaching and electric blending, in eggs, due to the antimicrobial protein in albumen that is released from egg yolk during stomaching and blending.

Mean values of microbial counts and pH values from different sampling methods of frozen blueberries at different dilutions are shown in Table 4.5. Mean values of YMC and pH showed differences ($p<0.05$) among sampling method. Stomaching had higher ($p<0.05$) YMC than massaging for both dilutions. Burnett and others (2004) reported that populations of *Listeria monocytogenes* were higher by stomaching than homogenizing, after inoculation on lettuce. Burnett and Beuchat (2001) also found that the population of *Salmonella* had higher recovery from stomaching on fresh blueberries. However, Ukuku and Fett (2004) reported

that blending had higher yeast and mold counts on unwashed whole cantaloupes.

pH values were different ($p<0.05$) among the three sampling methods and two dilutions for both fresh and frozen blueberries (Tables 4.4 and 4.5). Blending and homogenizing also had higher pH values than massaging. The pH values in the peptone solution tended to be higher before sampling. Organic acid in berries can be released to buffer solution, due to berry breakage during preparation and to ice crystals formed during freezing (result in cell membranes).

The pH values may be one of the reasons that caused microbial counts to be higher in massaged berries, because the organic acid and other antimicrobials from berries are released into the peptone solution. Beuchat and others (2001) claim that microbial growth may depend on different sampling method.

Because of the above reasons, massaging was used as the sampling (mixing) method on postharvest treatment experiments in this study.

Experiment III: Effect of disinfection treatments on fresh blueberry microbiological count and quality

Microbial Analysis

Table 4.6 shows the population of aerobic plate counts (APC) and yeast and mold counts (YMC) recovered from fresh blueberries by four different postharvest sanitation treatments. The APC were different ($P<0.05$) after postharvest treatment but not YMC ($p>0.05$). Chlorine at 400 ppm reduced APC by about 3 log CFU/g, but the other treatments did not have any effect. In a separate experiment, acidified sodium chlorite (600ppm, pH~2.3) reduced ($p\leqq0.05$) APC and YMC by 3.7 and 5 log CFU/g on fresh blueberries (Table 4.8). Similar results were found for frozen berries (Table 4.7), where APC was decreased ($p\leqq0.05$) in 400 ppm chlorine whereas YMC was not ($p>0.05$).

Yu and others (2001) reported that 200ppm chlorine could reduce 1.3 log *E. coli* O157:H7 compared with control. However, Crow and others (2005) found that 100 ppm chlorine for 30 sec could reduce yeast and mold counts after postharvest treatment on lowbush blueberries. The results from both fresh and frozen

blueberries indicate that 400 ppm but not 200 ppm chlorine can decrease APC, but only acidified sodium chlorite was able to decrease YMC in blueberries.

Costilow and others (1984) reported that microbial counts decreased in fresh cucumbers treated with 250ppm chlorine. Many postharvest fungicides can reduce yeast and mold counts (Ceponis and Cappellini 1978). Silva and others (1987) reported that if decay was more than 3.0%, Captan® fungicide was an effective postharvest treatment for increasing the shelf-life of berries stored at 4°C. However, Scholar® fungicide used in this study did not reduce microbial counts inculding yeast and mold counts. Dipping time and fungicide concentration may influence the results. Baillinger (1983) reported that blueberries dipped in high concentration of Captafol® for 30 min had a low percent decay. Moreover, kiwifruit dipped in the postharvest fungicide, Iprodione®, had a lower percentage botrytis stem-end rot compared when compared to untreated Kiwifruit (Pyke and others 1994).

Sensory Evaluation

Rating means of appearance, off-odor, off-flavor, and overall quality showed

no difference ($p>0.05$) among the four postharvest treatments for fresh blueberries (Table 4.9). The only significant difference ($p<0.05$) was skin color of fresh blueberries. Moreover, all of sensory evaluation ratings on appearance, skin color, off-odor, off-flavor, and overall quality showed no difference ($p>0.05$) in blueberries after 7 days of frozen storages (Table 4.10).

In addition, 60% of the panelists thought that fresh blueberries treated with 200 ppm chlorine were different compared with untreated blueberries (Data not presented). Half of the panelists thought fresh blueberries treated with tap water were not different from untreated berries. On the other hand, 76% of panelists thought that frozen blueberries treated with fungicide were different compared with untreated frozen berries, while 34% of panelists thought that blueberries treated with 200ppm chlorine and tap water were not different compared with untreated berries (data not presented).

The scores for frozen berries were lower than fresh berries. Slow freezing was used to freeze blueberries before storage. However, ice crystals will grow during slow freezing. After thawing, it causes fruit to become soft, shrink, and cellular material leaks out (Fellows 2003).

Table 4.1 Hunter color values of blueberries that are harvested at varying maturity stages

Maturity Stage	Hunter color values					
	L	a	b	HUE[x]	Chroma[x] (SI)	ΔE[x]
Green	30.6[NS]	-3.4b	18.4a	100[NS]	19.2a	39.1a
Red	21.6	16.9a	4.9b	16.5	17.7a	27.9ab
Ripe	17.2	0.5b	-1.5c	288	1.7b	17.3b
Overripe	18.8	0.8b	-2.3c	289	2.8b	19.0b
C.V.(%)[y]	55.3	76.5	37.3	-1203.6	17.9	31.7
LSD[z]	22.9	5.4	3.4	121.3	3.5	15.4

a,b,c – means within columns followed by a different letter differ ($p \leqq 0.05$)

NS-means within columns are not significantly different ($p>0.05$)

x - Hue angle = Hue angle value, SI= chroma or saturation index, and ΔE = total color difference

y - C.V. (%): Coefficient of Variation

z - LSD: Least significant difference ($\alpha=0.05$)

Table 4.2 Soluble solid content (SSC), titratable acidity (TA), ratio of soluble solid to titratable acidity (SSC:TA), and pH at different maturity stages of blueberries

Maturity Stages	SSC[x] (%)	TA[x] (%)	SSC:TA[x] (%)	pH
Green	5.3 c	1.95 a	2.7 d	4.1a
Red	7.6 b	1.22 b	6.2 c	3.0b
Ripe	8.8 b	0.69 c	12.7 b	3.1b
Overripe	11.2 a	0.67 c	16.8 a	3.1b
C.V.(%)[y]	9.3	8.33	11.4	14.4
LSD[z]	1.4	0.17	2.1	0.9

a,b,c – means within columns followed by a different letter differ ($p \leqq 0.05$)

x- SSC= Soluble solid content, TA= titratable acidity, SSC:TA= ratio of soluble solid and titratable acidity

y - C.V. (%): Coefficient of Variation

z - LSD: Least significantly difference (p=0.05)

Table 4.3 Aerobic plate counts (APC) and yeast and mold counts (YMC) of blueberries at different maturity stages.

Maturity Stages	APC[x] (CFU/g)[w]	YMC[x] (CFU/g)[w]
Green	3.30 [NS]	3.50 b[d]
Red	2.85	3.25 bc
Ripe	2.46	3.07 c
Overripe	3.61	3.97 a
C.V.(%)[y]	24.71	6.01
LSD[z]	1.42	0.39

a,b,c - with the same letter in columns means no significant difference
NS-means within columns are not significantly different (p>0.05)
w- colony forming unit for each gram.
x- APC= Aerobic plate counts, YMC= Yeast and mold counts
y- C.V. (%): Coefficient of Variation

z- LSD: Least significant difference (α=0.05)

Table 4.4 Recovery of yeast and mold counts (YMC), aerobic plate counts (APC), and pH value of fresh blueberries as affected by sampling method and dilution

Sampling Methods	Dilution Factor					
	APC[x]		YMC[x]		pH	
	1:3[a]	1:10[a]	1:3	1:10	1:3	1:10
Stomaching	1.67 c	2.00b	3.74[NS]	3.83[NS]	4.1b	3.9b
Massaging	1.99 b	3.00a	4.05	3.45	5.4a	5.9a
Blending	2.06 b	2.00b	3.82	3.58	3.8b	3.9b
C. V. (%)[y]	13.18		13.91		11.85	
LSD[z]	0.23		0.92		0.95	

a,b,c – means within category followed by a different letter differ ($p \leqq 0.05$)

NS - means no significant difference ($p>0.05$)

x- APC= Aerobic plate counts, YMC= Yeast and mold counts

y- C. V.(%): Coefficient of Variation

z- LSD: Least Significant Difference($\alpha=0.05$)

Table 4.5 Recovery of yeast and mold counts (YMC), aerobic plate counts (APC), and pH value of frozen blueberries as affected by sampling method and dilution

Sampling Methods	Dilution Factor					
	APC[x]		YMC[x]		pH	
	1:3[a]	1:10[a]	1:3	1:10	1:3	1:10
Stomaching	2.62[NS]	2.20[NS]	3.91a	3.93a	3.2a	3.4b
Massaging	1.64	2.44	3.25a	3.45b	3.8a	4.0a
Blending	2.26	2.69	3.61ab	3.44ab	3.7a	3.8ab
C. V. (%)[y]	22.86		16.22		5.71	
LSD[z]	0.94		0.98		0.37	

a - means the ratio of blueberry to peptone water
NS - means no significant difference ($p<0.05$)
x- APC= Aerobic plate counts, YMC= Yeast and mold counts
y- C. V.(%): Coefficient of Variation
z- LSD: Least Significant Difference($p=0.05$)
a,b,c - with the same letter in columns means no significant difference

Table 4.6 Population of aerobic plate counts (APC) and yeast and mold counts (YMC) recovered from fresh blueberries after postharvest sanitation treatment.

Postharvest treatments	Population recovered counts (CFU/g)[d]			
	APC	Reduction[a]	YMC	Reduction[a]
Control(Dry)	3.87a	--	4.47[NS]	--
Tap Water	3.97a	-0.1	4.45	0.02
Scholar® Fungicide*	3.22a	0.65	4.31	0.16
200ppm Chlorine	2.50ab	1.82	3.64	0.83
400ppmChlorine*	1.00b[e]	2.87	3.88	0.59
C.V.(%)[b]	12.74		10.73	
LSD[c]	0.767		0.84	

a- present reduction was determined by: $Population_{control}$ - $population_{treated}$

b- C. V.(%): Coefficient of Variation

c- LSD: Least Significant Difference(α=0.05)

d- CFU: Colony Forming Unit

e- When microbial count <25, it record as 1 in this study.

NS- means no significant difference ($p<0.05$)

a,b,c – means within column with the same letter are not different ($p<0.05$)

* Berries from those treatments were rinsed by tap water after washing.

Table 4.7 Population of aerobic plate counts (APC) and yeast and mold counts (YMC) recovered from frozen blueberries after 7 days of storage by four different postharvest sanitation treatments and untreated.

Postharvest treatments	Population recovered counts (CFU/g)[d]			
	APC	Reduction[a]	YMC	Reduction[a]
Control (untreated)	3.86a	--	4.13[NS]	--
Water	3.54ab	0.32	4.04	0.09
Scholar® Fungcide	3.19ab	0.67	4.42	-0.29
200ppm Chlorine	1.87ab	1.99	3.78	0.26
400ppm Chlorine	1.59b	2.27	3.61	0.97
C.V. (%)[b]	21.70		11.88	
LSD[c]	1.20		0.89	

a- present reduction was determined by: control CFU/g – treated CFU/g

b- C. V.(%): Coefficient of Variation

c- LSD: Least Significant Difference (p=0.05)

d- CFU: Colony Forming Unit

a,b,c - with the same letter in columns means no significant difference

NS- means no significant difference (p<0.05)

Table 4.8 Aerobic plate counts (APC) and yeast and mold counts (YMC) recovered from fresh and frozen blueberries after 600 ppm acidified sodium chlorite for 30 sec

Control & Treated		Microbial counts (CFU/g)[d]	
		APC	YMC
Fresh	Control	3.75 ± 0.44 a	5.02 ± 0.29 a
	Treated	< 1.4[b] b	< 1.4[b] b
Frozen	Control	3.2 ± 0.14 A	4.69 ± 0.28 A
	Treated	< 1.4[b] B	< 1.4[b] B
	C.V. (%)[b]	12.95	0.12
	LSD[c]	0.881	0.01

a,b, - with the same letter in columns means no significant difference

A, B- with the same letter in columns means no significant difference

a- CFU: Colony Forming Units/ garm of berries

b- <25 means no colonies found on plate at 10^{-2} and it was represent 1 for data analysis

Table 4.9 Sensory evaluation ratings of appearance, skin color, off-odor, off-flavor, and overall quality of fresh blueberries after four different postharvest treatments.

Postharvest treatments	Appearance*	Skin color *	Off-odor *	Off-flavor*	Overall quality*
Water	3.47^{NS}	3.77ab	3.77^{NS}	3.63^{NS}	3.00^{NS}
Scholar® Fungcide	3.37	4.00a	3.90	3.57	2.87
200ppm Chlorine	3.07	3.70b	3.73	3.57	2.93
400ppm Chlorine	3.26	3.80ab	3.77	3.43	2.93
C.V.(%)[a]	12.68	3.12	4.80	7.29	15.61
LSD[b]	0.83	0.24	0.36	0.52	0.91

a- C. V.(%): Coefficient of Variation

b- LSD: Least Significant Difference (p=0.05)

NS- means no significant difference (p<0.05)

a,b,c - with the same letter in columns means no significant difference

* Ratings were assigned by panelists using 1 to 5 scales with 1 indicate shrink and 5 indicating extreme plump, and firm for appearance, 1 indicate red color and 5 indicate extreme deep purple for skin color, 1 indicate chemical and 5 indicate extreme fresh and none for off-odor, 1 indicate spooled chemical and 5 indicate extreme fresh and sweet for off-flavor, and 1 indicate dislike extremely and 5 indicate like extremely for overall quality.

Table 4.10 Sensory evaluation ratings to appearance, skin color, off-odor, off-flavor, and overall quality on frozen blueberries after 7 days storage by four different postharvest treatments.

Postharvest Treatments	Appearance*	Skin color*	Off-odor*	Off-flavor*	Overall quality*
Water	2.13^{NS}	2.80^{NS}	2.83^{NS}	2.77^{NS}	2.60^{NS}
Scholar® Fungcide	1.83	2.77	2.93	2.50	2.20
200ppm Chlorine	2.13	2.80	2.90	2.43	2.33
400ppm Chlorine	2.13	2.67	2.77	2.63	2.67
C.V.(%)[a]	13.81	8.71	7.40	15.71	11.50
LSD[b]	0.57	0.48	0.43	0.81	0.54

a- C. V.(%): Coefficient of Variation

b- LSD: Least Significant Difference (p=0.05)

NS- means no significant difference (p<0.05)

* Ratings were assigned by panelists using 1 to 5 scales with 1 indicate shrink and 5 indicating extreme plump, and firm for appearance, 1 indicate red color and 5 indicate extreme deep purple for skin color, 1 indicate chemical and 5 indicate extreme fresh and none for off-odor, 1 indicate spooled chemical and 5 indicate extreme fresh and sweet for off-flavor, and 1 indicate dislike extremely and 5 indicate like extremely for overall quality.

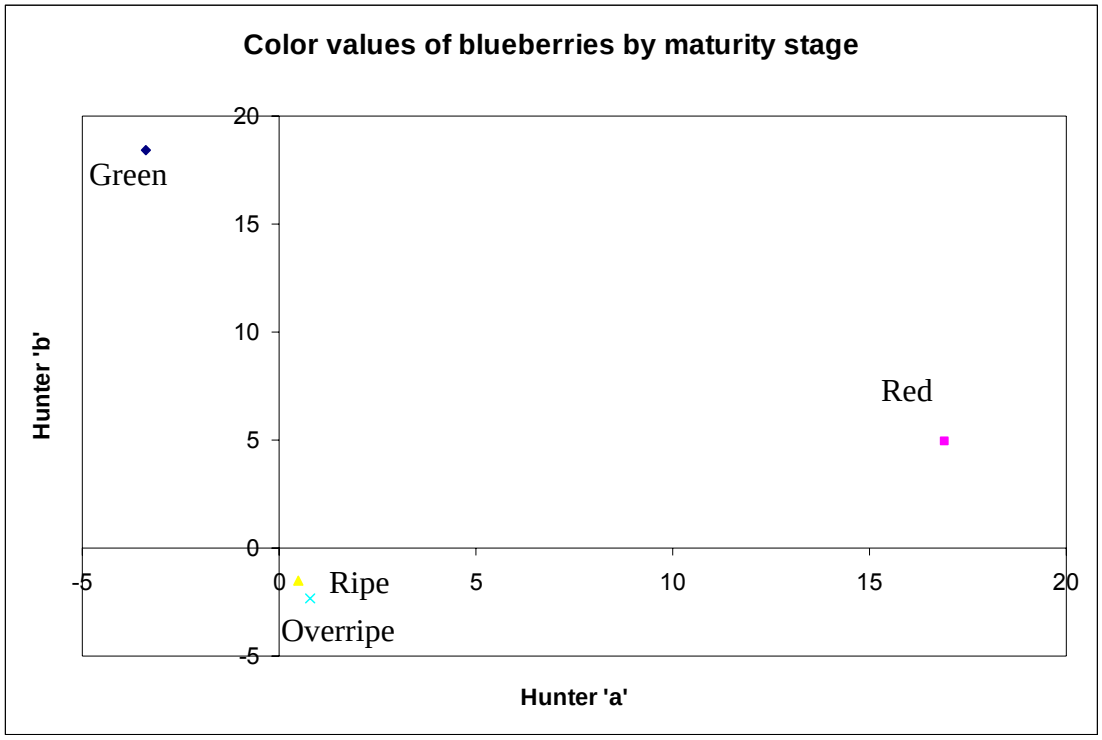

Figure 4.1 Color locations of blueberries at four different maturity stages on Hunter color scale

CHAPTER V

SUMMARY AND CONCLUSIONS

When blueberries are fully ripe, they have a deep purple color. Blueberries have lower microbial counts and are sweeter at this stages. Although there were no difference in aerobic plate counts, yeast and mold counts are lower when blueberries were ripe, and increased when overripe. Massaging of the fresh berries in 1:10 dilution resulted in higher aerobic plate counts while stomaching and blending of frozen berries resulted in higher yeast and mold counts. Chlorine 400 ppm showed a significant reduction on APC, while acidified sodium chloride reduced APC and YMC to non detectable levels.

It is recommended that scientists massage berries, especially fresh berries, when conducting microbial count. Furthermore, it is recommended that packing houses validate their fruit sanitation procedures to insure an effective reduction in

microbial counts and thus meet market demands and shelf life requirements. However, maturity stages at harvest my influence the microbial load on the berries.

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APPENDIX

Table A.1 Population of Aerobic plate counts (APC) and Yeast and molds counts (YMC) recovered from machine harvest blueberries treated with different postharvest sanitation treatments.

Postharvest Treatments	Population recovered counts (CFU/g)[b]			
	APC	Reduction[a]	YMC	Reduction[a]
Control	3.4	--	5.33	--
20% Ethanol	2.89	0.51	5.21	0.12
70% Ethanol	2.79	0.61	5.29	0.04
100 ppm Chlorine	3.17	0.23	5.36	-0.03
200 ppm chlorine	3.43	-0.03	5.39	-0.06
25 ppm Hydrogen Peroxide	3.66	-0.26	5.58	-.0.25
50 ppm Hydrogen Peroxide	3.8	-0.4	5.46	-0.13

a- present reduction was determined by: control CFU/g – treated CFU/g

b- CFU: Colony Forming Unit

Table A. 2 Population of Aerobic plate counts (APC) and Yeast and molds counts (YMC) recovered from hand harvest blueberries treated with different postharvest sanitation treatment.

Postharvest Treatments	Population recovered counts (CFU/g)[b]			
	APC	Reduction[a]	YMC	Reduction[a]
Control	3.14	--	4.15	--
20% Ethanol	3.16	-0.02	5.04	-0.89
70% Ethanol	3.57	-0.43	3.7	0.45
100 ppm Chlorine	2.42	0.72	4.27	-0.12
200 ppm chlorine	3	0.14	4.53	-0.38
25 ppm Hydrogen Peroxide	2.55	0.59	4.44	-0.29
50 ppm Hydrogen Peroxide	2.65	0.49	5.02	-0.87
Tasker	2.85	0.29	4.7	-0.55
Ozone	2.57	0.57	4.41	-0.26

a- present reduction was determined by: control CFU/g – treated CFU/g

b- CFU: Colony Forming Unit

Table A.3 Population of Aerobic plate counts (APC) and Yeast and mold counts (YMC) from store sample on fresh blueberries sampled during 2005-2006

Production place or country	Test date	Microbial counts (CFU/g)[a]	
		APC	YMC
Snnyridge--Argentina	December, 2005	4.3	1
		4.1	2
		4.3	1
		4.2	3
Nice Berry®--Argentina	December, 2005	1	4.39
		2	4.46
		2.6	4.7
		2.5	4.88
Snnyridge--Florida	July, 2005	2.1	4.51
		2	4.2
Blueberries Heritage--Michigan	July, 2005	3.11	4.9
		4.8	4.5
		2.8	5
Bonnie Blue®--North Carolina	July, 2005	2.8	5.2
		3.2	4.8
		3.4	4,8
		3.5	4.88
		2.54	4.47
Snnyridge—North Carolina	July, 2005	4.17	5.23
		3.84	5.26
Snnyride--Georgia	July, 2005	4.16	>300
		4.2	>300
Naturipe®--Great Lake	August, 2005	2.6	4.53
		3.07	5.22
		2	4.06
		1	3.96

Table A.3 (Cont.) Population of Aerobic plate counts (APC) and Yeast and mold counts (YMC) from store sample on fresh blueberries sampled during 2005-2006

Production place or country	Test date	Microbial counts (CFU/g)[a]	
		APC	YMC
Snnyridge--Canada	August, 2005	2.77	4.97
		3.23	4.89
Snnynidge--Georgia	September, 2005	3.27	5.1
		3.04	5.6
AF	January, 2006	2.87	4.55
Naturipe	January, 2006	1	4.02
Cottle Farm-- Chile	January, 2006	1	3.4
Naturipe--Chile	January, 2006	2	4.54
Global berries farm--Florida	April, 2006	4.3	5.04
		4.2	5
Wild Blueberries(lowbush), ME	--	3.14	5.72
		3.41	5.36
		3.38	5.23
Fresh harvest Pompano beach, Florida, Mississippi berries	June, 2006	4.43	5.08
Snnyridge--Canada	August, 2006	4.5	5.06
Michigan Summer Blueberries, Michigan	September, 2006	3.2	4.3
Naturipe®--Great Lake®--USA	September, 2006	3.6	3.6
A &L Farms Inc. USA	September, 2006	4.8	5.52

Table A.4 Population of Aerobic plate counts (APC) and Yeast and molds counts (YMC) from store sample on frozen blueberries sampled during 2005-2006

Production	Test date	Microbial counts (CFU/g)[a]	
		APC	YMC
Wal-mart Great Value	January, 2006	1	4.3
Southern Home		2	3.87
Premium Quality Private Selection®		3.16	4.02
Wild blueberries(Lowbush), ME	--	3.41	5.02
		2.3	4.91
		2.47	4.96
Premium Quality Private Selection®	March, 2006	1	3.07
Wal-mart Great Value	March, 2006	1	3.91

Table A.5 Population of Aerobic plate counts (APC) and Yeast and molds counts (YMC) from TRAICOFF from Mississippi

Source	Stage of Berry	Date of Harvest	Microbial Count (CFU/g) APC	YMC
TRAICOFF	Fresh	July, 2005	3.43	4.56
			3.61	4.44
			3.19	4.43
Mean ± SD			3.41± 0.21	4.48 ± 0.07
TRAICOFF	Frozen	July, 2005	2.47	4.14
			2.00	3.69
			3.14	4.11
Mean ± SD			2.54 ± 0.57	3.98 ± 0.25